MYCOTAXON

ISSN (print) 0093-4666 (online) 2154-8889 Mycotaxon, Ltd. ©2018

April–June 2018—Volume 133, pp. 307–313

https://doi.org/10.5248/133.307

Notes on rust fungi in China 5. Hosts and distribution of *Uromyces gageae* and its intracellular spermogonia

Jing-Xin Ji[1], Zhuang Li[2], Yu Li[1], Makoto Kakishima[1, 3*]

[1]*Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, Changchun, Jilin 130118, China*

[2]*College of Plant Pathology, Shandong Agricultural University, Tai'an 271000, China*

[3]*University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan*

* Correspondence to: *kakishima.makoto.ga@u.tsukuba.ac.jp*

Abstract—A rust fungus on *Gagea nakaiana* was collected in Jilin Province, northeast China. Based on morphological observations, specimens on this plant were identified as *Uromyces gageae*. This plant is a new host for *U. gageae* in China and the rust is newly recorded from northeast China. During field investigations orange-yellow spots were frequently observed on the leaves with rust sori. Molecular and morphological analyses confirm that those spots represent spermogonia of *U. gageae* produced in host cells.

Key words—*Liliaceae, Pucciniomycetes,* taxonomy, *Uredinales*

Introduction

Gagea nakaiana [≡ *G. lutea* var. *nakaiana*] is a perennial herb producing yellow flowers in early spring, the plant disappearing after the flowers have died. *Gagea nakaiana* is distributed in northeast China, Japan, Korea, Russian Far East, Nepal, Pakistan, Sikkim, and India (Flora of China, www.eFloras.org). There have been no previous reports of rust fungi on *G. nakaiana* in China (Tai 1979, Zhuang 2005), but in May 2016 during our investigations of rust fungi in Jilin Province, northeast China, the telial stage of a rust fungus was found on *G. nakaiana*. The rust was identified through morphological observations of the Jilin Province specimens.

Orange-yellow spots around telial sori on the leaves were also frequently observed in the specimens. These spots, which contained numerous small spermatium-like spores, were suspected to represent spermogonia of the rust fungus. Inoculation experiments with teliospores are difficult because leaves with telia are found only in early spring, and teliospores are contaminated with soil from around plants. We report here the results of our morphological observations and molecular analyses of the rust to confirm the relationship between these suspected spermogonia spots and telia.

Materials & methods

Specimens on *G. nakaiana* were collected from the forest floor in Jilin, Jilin Province, China, in May 2016 and April 2017. The deciduous broad-leaved forest, typical for the area, is composed primarily of *Betula, Populus,* and *Quercus* species. The specimens used for morphological observations and molecular analysis were deposited in the Herbarium of Mycology, Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, China (HMJAU).

Morphological characters (including the size and shape of sori and spores) were examined using light (LM) and scanning electron microscopy (SEM) as reported in Ji & al. (2017).

For molecular analyses total genomic DNA was extracted separately from a mass of spermatium-like spores from a single orange-yellow spot and about 200 spores from a single telium. Orange-yellow spots and telia were selected from separate isolated areas on the leaves to avoid cross contamination. Spores were crushed between two sterilized glass slides and suspended in 30 µl extraction buffer [10 mM Tris-HCl pH 8.3, 1.5 mM MgCl2, 50 mM KCl, 0.01% sodium dodecyl sulfate (SDS), 0.01% Proteinase K], and the suspensions were incubated at 37°C for 1 hour and 95°C for 10 min, followed by a 4°C soak (Suyama & al. 1996, Virtudazo & al. 2001). From the crude extract, 5–7 µl samples were used directly for each polymerase chain reaction (PCR). The rDNA-28S region was amplified using primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) (O'Donnell 1993), and the rDNA-ITS region was amplified using primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) (Gardes & Bruns 1993) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White & al. 1990). The DNA was amplified in a 50 µl mixture containing 5 µl of template DNA, 200 µl of each primer, 25 µl of Premix TaqTM (TaKaRa TaqTM Ver. 2.0 plus dye), and 18 µl of ddH_2O. Amplification cycling conditions consisted of 94°C for 5 min, followed by 35 denaturation cycles at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min, and a final extension at 72°C for 10 min. PCR products were separated on 1% agarose gels containing Nucleic Acid Stain (Beijing Dinggou Changsheng Biotechnology Co.) and purified using the TaKaRa MiniBEST Agarose

Fig. 1. *Uromyces gageae* on *Gagea nakaiana*. A. *Gagea nakaiana* with yellow flowers; B. Orange-yellow spots of spermogonia with sweetish exudates on a leaf; C. Vertical section of a spermogonium produced in a hypertrophied host epidermal cell; D. Dark brown telia on a leaf; E. Teliospores with hyaline papilla; F. Vertical section of a telium. Scale bars: C, E = 15 μm; F = 20 μm.

Gel DNA Exaction Kit Ver.4.0. Purified PCR products were cloned in pEASY®-T1 Cloning Vector and then transferred into Trans1-T1 phage, resistant chemically competent cell according to the Transgene Biotech instructions. The positive clones

were sequenced by Sangon Biotech Co. in Shanghai. All data sequenced in this experiment were deposited at GenBank.

Results & discussion

The telia and teliospores on *G. nakaiana* were identified as *Uromyces gageae* based on their morphological similarity to the descriptions of *U. gageae* by Gäumann (1959), Wilson & Henderson (1966), Hiratsuka & al. (1992), Azbukina (2005), and Termorshuizen & Swertz (2011), although the position of telia on the leaves and teliospore sizes differed somewhat among the descriptions. These variations may be attributable to differences in *U. gageae* populations and in host plants, given the wide range of both the rust and its host plants.

The orange-yellow leaf spots were superficially similar to spermogonia of rust fungi because of sweetish exudates from abundant minute orange-yellow spots (Fig. 1B). Spherical to hemispherical fungal structures containing small spermatia-like spores were observed inside hypertrophied epidermal cells of the leaves (Fig. 1C). Hyphal structures, including receptive hyphae, were not observed around these structures. These orange-yellow spots were also reported by Plowright in 1889, although the function of these spots was not clarified (cited from Wilson & Henderson 1966). Therefore, we conducted molecular analyses to confirm the relationship between these spots and telia of the rust fungus. Sequence data of rDNA-ITS and 28S regions were successfully obtained from single orange-yellow spots (HMJAU8557, GenBank MG742206 [ITS]; HMJAU8559, GenBank MG742208 [28S]) and single telia (HMJAU8558, GenBank MG742207 [ITS]; HMJAU8560, GenBank MG742209 [28S]). Sequence similarities of the ITS (796 bp) and 28S (652 bp) regions between the orange-yellow spot and the telium were 100%, thus confirming that the spots and telia are genetically identical and that these spots on *G. nakaiana* represent structures belonging to the rust fungus. We believe that the spots are spermogonia, although the structures differ in shape and position within the leaf tissue from previously described spermogonia (Hiratsuka & Cummins 1963, Cummins & Hiratsuka 2003), and their function is still not confirmed.

A description of the morphological features obtained from specimens on *G. nakaiana* collected in China follows.

Uromyces gageae Beck, Verh. Zool.-Bot. Ges. Wien 30: 26, 1880. Figs 1, 2

Spermogonium-like structures produced inside of hypertrophied cells amphigenous, minute, gregarious, orange-yellow, spherical to

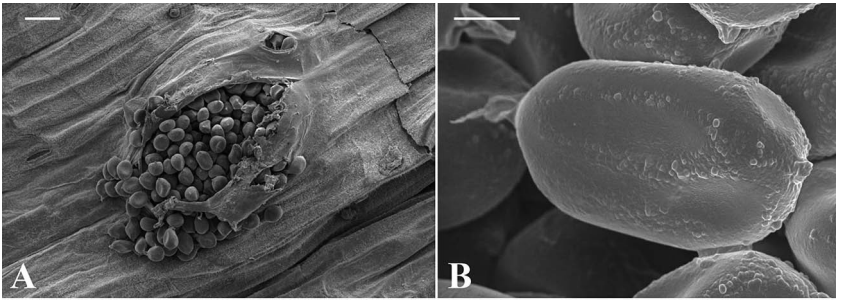

Fig. 2. *Uromyces gageae* on *Gagea nakaiana*. Telia on a leaf (A) and teliospores with small irregular verrucae (B) observed by SEM. Scale bars: A = 50 μm; B = 10 μm.

hemispherical. Aecia and uredinia not found. Telia amphigenous, dark brown to black, scattered on leaves, roundish or ellipsoid, first covered by epidermis, pulverulent, dark brown. Teliospores ellipsoid, ovate to globose, 27–39 × 19–25 μm (av. 33 × 22 μm); apex rounded with conical, hyaline papilla (ca. 2 μm); walls brown, mostly smooth, ca. 1.0–2.5 μm (av. 1.5 μm) thick, small irregular verrucae observed by SEM; pedicels hyaline, deciduous, short.

Specimens examined—Spermogonium-like structures and telia on *Gagea nakaiana* Kitag. (*Liliaceae*): **CHINA: Jilin Province**, **Jilin**, Jiaohe, Hongyegu, 43°42′13″N 127°04′18″E, alt. 535 m, 13 May 2016, (HMJAU8557, GenBank MG742206; HMJAU8558, GenBank MG742207); Zuojia, 44°03′51″N 126°05′54″E, alt. ca. 500 m, 21 April 2017 (HMJAU8559, GenBank MG742208); 30 April 2017 (HMJAU8560, GenBank MG742209).

Hosts & distribution in china—*Gagea nakaiana* [≡ *G. lutea* var. *nakaiana* (Kitag.) Q.S. Sun], Jilin Province; *Gagea triflora* (Ledeb.) Schult. & Schult.f. [≡ *Lloydia triflora* (Ledeb.) Baker], Inner Mongolia.

Uromyces gageae has been reported on *Gagea arvensis* (Pers.) Dumort., *G. bohemica* (Zauschn.) Schult. & Schult.f., *G. liottardii* (Sternb.) Schult. & Schult.f., *G. lutea* (L.) Ker Gawl., *G. pusilla* (F.W. Schmidt) Sweet, *G. soleirolii* F.W. Schultz, *G. spathacea* (Hayne) Salisb., and *Lloydia triflora* from Europe (Gäumann1959, Wilson & Henderson 1966, Termorshuizen & Swertz 2011); on *G. nakaiana*, *G. pauciflora* (Turcz. ex Trautv.) Ledeb., and *L. triflora* from Russian Far East (Azbukina 2005); and on *G. lutea*, and *L. triflora* from Japan (Hiratsuka & al. 1992). This rust was reported from China for the first time on *L. triflora* from Inner Mongolia (Yang & al. 2018), and *G. nakaiana* is reported here as a new host plant for China. Because *Gagea* species are

widely distributed in northern China, we suspect that *U. gageae* is also widely distributed in the area.

In our field survey, *U. gageae* was observed to produce orange-yellow spots and telia on the new young leaves when they appeared in early spring; these sori became scattered over the whole leaf surface as the leaves expanded. After producing flowers and seeds in early spring, the plant disappears until next year. However, *U. gageae* produces sori on the same individual plants in every year. We therefore suspect that *U. gageae* survives in the plant bulbs and systemically infects the new leaves as they emerge.

Acknowledgments

This work was financed by the Fungal Flora in Jilin Province (20130206073NY). We wish to thank Dr E.H.C. McKenzie (Manaaki Whenua Landcare Research, Auckland, New Zealand) and Dr C.M. Denchev (Bulgarian Academy of Sciences, Sofia, Bulgaria) for critical reading of the manuscript and suggestions.

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